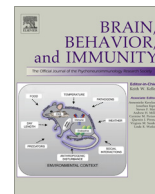




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Gender differences in the association of sleep apnea and inflammation

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ABSTRACT

Over the last 15 years, many studies have established an association of sleep apnea with inflammation and metabolic aberrations. However, no controlled studies have examined potential gender effects in this association. We recruited 120 middle-aged, predominantly non-obese mild-to-moderate sleep apneics and controls (62 males, 58 females). All participants underwent a clinical history, physical examination, and 1-night 8-h polysomnography recording and provided a single fasting blood sample for assessment of interleukin-6 (IL-6), tumor necrosis factor receptor 1 (TNFR1), C-reactive protein (CRP), leptin, and adiponectin levels. Among non-sleep apneics, females had higher levels of TNFR1 ($p = 0.01$), CRP ($p = 0.005$), leptin ($p < 0.001$), and adiponectin ($p < 0.001$) compared to males, independent of age and body mass index. When analyzed separately by gender, sleep apneic men had elevated TNFR1 ($p = 0.04$), CRP ($p = 0.06$) and IL-6 ($p = 0.11$) relative to control men; in sleep apneic females, only CRP was elevated ($p = 0.04$). Furthermore, CRP was associated with apnea severity in a dose-response manner (p -linear = 0.04 in both genders) and was independently associated with comorbid hypertension in apnea (p -linear = 0.005 for women; p -linear = 0.09 for men). In conclusion, although women have naturally higher levels of inflammatory and metabolic markers than men, sleep apneic men appear to have a more severe inflammatory profile compared to women. Our findings suggest that these markers should be analyzed and interpreted separately in men and women, and that a single measure of plasma CRP appears to be a clinically-useful marker of apnea severity and comorbid cardiovascular morbidity.

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1. Introduction

Obstructive sleep apnea is a prevalent sleep disorder characterized by obstruction of the upper airway during sleep despite breathing effort, as well as an associated reduction in blood oxygen saturation. Seventeen to 24% of men and 5–9% of women in general population samples demonstrate an apnea-hypopnea index (AHI) of five or more events per hour of sleep, while 4% of men and 2% of women meet the current clinical and polysomnographic criteria for the diagnosis of sleep apnea warranting immediate therapeutic intervention (Bixler et al., 1998, 2001; Young et al., 1993). Sleep apnea has been associated with the elevation of several pro-inflammatory cytokines, independent of obesity (Kritikou et al., 2014; Sahlman et al., 2010; Shamsuzzaman et al., 2002; Vgontzas et al., 1997, 2000, 2008). Among a number of inflammatory

pathways, sleep apnea has been especially linked to activation of tumor necrosis factor (TNF)- α receptors, which stimulates secretion of interleukin (IL)-6, in turn triggering the synthesis of C-reactive protein (CRP) in the liver (Akira et al., 1990; Hirano et al., 1990). It is hypothesized that this inflammatory cascade, in addition to insulin resistance, mediates the link between sleep apnea and cardiometabolic complications (Vgontzas et al., 2005a).

Although sleep apnea was traditionally recognized in middle-aged, obese men, its occurrence in women as well as lean individuals is increasingly recognized. The prevalence of sleep apnea increases markedly after menopause, with post-menopausal women having a doubled rate of apnea compared to pre-menopausal women, even after accounting for neck circumference and body mass index (Bixler et al., 2001; Dancsey et al., 2001). Also, while the maximum prevalence for obstructive sleep apnea peaks between ages 50 and 59 in men (Bixler et al., 1998), this peak is not seen in females until after age 65 (Bixler et al., 2001). Furthermore, men tend to have a higher AHI than women when matched for body mass index (Kapsimalis and Kryger, 2002), are more likely to exhibit the classical symptoms of excessive daytime sleepiness

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and snoring (Phillips et al., 2008), and the severity of their daytime sleepiness is more likely to be related to lack of regular exercise, depression, and minimum oxygen desaturation than AHI per se (Basta et al., 2008).

In healthy individuals, it has been demonstrated that peripheral levels of CRP (Cartier et al., 2009; Khera et al., 2005; Lakoski et al., 2006; McConnell et al., 2002; Wener et al., 2002), leptin (Couillard et al., 1997; Hellström et al., 2000; Hickey et al., 1996; Kennedy et al., 1997; Ostlund et al., 1996), and adiponectin (Böttner et al., 2004; Saltevo et al., 2009; Song et al., 2014) are naturally higher in women compared to men, independent of age, race, and body mass index. Despite this, although most studies of sleep apneics statistically control for gender in their analyses, very few have expressly investigated possible gender differences in inflammation.

A number of studies in large general population samples, both prospective and cross-sectional, have demonstrated a clear association between sleep apnea and hypertension (Bixler et al., 2000; Nieto et al., 2000; Peppard et al., 2000). Subsequent studies in large community-based cohorts have further demonstrated that men with sleep apnea have a significantly increased risk for hypertension (Hedner et al., 2006; Mohsenin et al., 2009) and stroke (Redline et al., 2010) compared to women. Additionally, a recent large study reported that men with serum CRP > 3.0 mg/L have significantly higher odds of both cardiovascular and all-cause mortality compared to women with the same CRP cut-off (Doran et al., 2013). However, no study to date has explored the link between gender and apnea-associated cardiovascular outcomes, such as hypertension, in the context of the inflammatory response.

The aim of our study was to examine potential gender differences in the association of sleep apnea with inflammation and metabolic markers, as well as the synergistic effect of apnea and hypertension on these outcomes in a sample of middle-aged, predominantly non-obese sleep apneics and controls. We hypothesized that the association of these markers with sleep apnea would be stronger in men than in women.

2. Methods

2.1. Participants

The study sample consisted of 120 middle-aged, predominantly non-obese mild-to-moderate sleep apneics and controls (62 males, 58 females; mean age = 54.67 ± 0.54 years). Participants were recruited through advertisements in the local community and screened according to research protocols by the Sleep Research and Treatment Center at Penn State Milton S. Hershey Medical Center (Hershey, PA, USA). All women in the study were post-menopausal (self-reported absence of menses for at least 12 months or total hysterectomy). Exclusion criteria included a history of diabetes mellitus, use of antidiabetic agents and/or fasting blood glucose levels >126 mg/dL, ongoing infections, rheumatoid arthritis, insomnia, narcolepsy, and use of certain medications (psychotropics, steroids, sympathomimetics, sympatholytics, or hormone therapy for females). The study was approved by the Institutional Review Board at Penn State University College of Medicine and all participants provided written informed consent.

2.2. Sleep laboratory protocol

During their visit in the laboratory, all participants underwent a clinical history and physical examination, during which height and weight were recorded and body mass index (BMI) calculated (in kg/m²). Blood pressure was also assessed, with hypertension defined as systolic blood pressure ≥ 140 mmHg and/or diastolic blood pressure ≥ 90 mmHg, or as the use of antihypertensive medication.

Sleep laboratory recordings were conducted in a sound-attenuated, light- and temperature-controlled room with a comfortable, bedroom-like atmosphere. Each subject was monitored continuously for one night for 8 h (22:30–23:00 until 6:30–7:00) using 16-channel polygraph recordings of EEG, electrooculogram (EOG) and electromyogram (EMG). Polysomnography (PSG), respiration (via thermocouple and thoracic strain gauges), and oximeter data were collected using Grass-Telefactor Gamma Sleep Recording software (Middleton, WI, USA). Visual sleep stage scoring was conducted by a registered polysomnography technologist blind to participant characteristics based on Rechtschaffen and Kales criteria (Rechtschaffen and Kales, 1968). Apnea-hypopnea index (AHI; number of apneas and hypopneas summed per hour) was also ascertained. An apnea was defined as cessation of airflow for ≥ 10 s and an out-of-phase strain gauge movement; a hypopnea was defined as a 50% airflow reduction and associated decrease in SaO₂ of at least 4%. In stratifying our study sample, “the presence of sleep apnea” was defined as an AHI ≥ 5 events/hour of sleep.

2.3. Blood sampling

A single fasting blood draw (via venipuncture) was performed at 7:00 immediately after the end of the PSG recording. Blood was collected in EDTA-containing tubes and refrigerated until centrifugation (within 3 h). Blood was stored at -80°C until assay.

2.4. Assays

Plasma interleukin-6 (IL-6), tumor necrosis factor receptor 1 (TNFR1), and high-sensitivity C-reactive protein (hsCRP) were measured via enzyme-linked immunosorbent assay (ELISA) (R&D Systems, Minneapolis, MN). The intra- and inter-assay coefficients of variation (CVs) were 7.4% and 7.8% for IL-6, 4.4% and 6.1% for TNFR1, and 5.5% and 11.6% for hsCRP. The lower detection limits were 0.094 pg/mL, 0.043 pg/mL, and 0.124 ng/mL for IL-6, TNFR1, and hsCRP, respectively. Leptin and adiponectin were assessed by commercially-available radioimmunoassays with CVs below 10%.

2.5. Statistical analysis

Two-tailed independent-samples t-tests were used to compare demographic and PSG variables between males and females (between-gender), or between controls and sleep apneics (within-gender). To examine differences in inflammatory and metabolic characteristics between more than two groups (e.g. increasing apnea severity), analyses of covariance (ANCOVA) with Bonferroni correction were conducted. Polynomial linear analysis was also performed to examine the association between increasing apnea severity (i.e. AHI < 5, $5 \leq \text{AHI} < 15$, and AHI ≥ 15) and inflammatory markers. Finally, to assess the association of sleep apnea associated with the comorbid cardiovascular outcome (i.e., hypertension) and inflammation, we examined differences between three groups: controls without hypertension, sleep apneics without hypertension, and sleep apneics with hypertension. Effect size was also assessed by calculating Cohen's d statistic. Statistical significance was determined using the criterion $p < 0.05$. All analyses were adjusted for the confounders age and BMI. Analyses were conducted using the Statistical Package for the Social Sciences (SPSS) version 22.0 (IBM Corp., Armonk, NY).

3. Results

Demographic and PSG parameters of sleep apneic and control males and females are presented in Table 1. Within gender, the control and sleep apneic groups did not differ in age (all $p > 0.05$), but

sleep apneics tended to have higher BMI (27.79 kg/m² vs. 26.50 kg/m² respectively, $p = 0.08$ for males; 31.09 kg/m² vs. 28.37 kg/m², $p = 0.03$ for females) and waist circumference (100.54 vs. 96.03 cm respectively, $p = 0.03$ for males; 99.10 vs. 92.33 cm respectively, $p = 0.04$ for females) than controls. A larger neck circumference was also observed in males with sleep apnea compared to controls (39.87 cm vs. 38.22 cm, $p = 0.04$), but not in females ($p = 0.60$). Furthermore, systolic blood pressure was significantly elevated in females with sleep apnea (134.52 mmHg vs. 120.24 mmHg, $p = 0.002$), but this difference was not observed for diastolic blood pressure, nor any blood pressure measures in men (all $p > 0.15$). In this sample, controls and sleep apneics did not differ significantly in any sleep efficiency or architecture parameters (Table 1).

Inflammatory and metabolic characteristics of controls and sleep apneics stratified by gender are presented in Table 2. Control females had significantly higher levels of TNFR1 (1.23 ng/mL vs. 0.97 ng/mL respectively; $p = 0.01$), CRP (1.83 ng/mL vs. 0.90 ng/mL, $p = 0.005$), leptin (28.52 ng/mL vs. 6.83 ng/mL; $p < 0.001$) and adiponectin (13.38 ng/mL vs. 5.70 ng/mL; $p < 0.001$) compared to control males. These gender differences were seen in sleep apnea as well; female apneics had significantly higher levels of CRP (2.81 ng/mL vs. 1.56 ng/mL, $p = 0.002$), leptin (32.96 ng/mL vs. 5.54 ng/mL, $p < 0.001$), adiponectin (11.53 ng/mL vs. 5.13 ng/mL, $p < 0.001$), and a trend toward higher TNFR1 (1.26 ng/mL vs. 1.13 ng/mL, $p = 0.08$) compared to apneic males.

Because of these basal gender differences, the remaining analyses were conducted separately in males and females (within-gender). Sleep apneic males had a significantly higher concentration of plasma TNFR1 than controls (1.13 ng/mL vs. 0.97 ng/mL; $p = 0.04$) and a trend towards higher CRP (1.56 ng/mL vs. 0.90 ng/mL; $p = 0.06$) and IL-6 (1.14 pg/mL vs. 0.82 pg/mL; $p = 0.11$). In females, only CRP was elevated in sleep apneics compared to controls (2.81 ng/mL vs. 1.83 ng/mL; $p = 0.04$) (Table 2).

We then examined the association of increasing apnea severity (AHI < 5, 5 ≤ AHI < 15, and AHI ≥ 15) with inflammatory markers in men and women. A dose-response was observed with CRP in both genders (p -linear = 0.04; Fig. 1). Men with AHI ≥ 15 ($n = 27$) had significantly higher CRP than control men ($n = 15$; 1.67 ng/mL vs. 0.90 ng/mL respectively; $p = 0.04$; Cohen's $d = 0.69$). Similarly, women with AHI ≥ 15 ($n = 20$) had significantly higher CRP than control women ($n = 23$; 2.96 ng/mL vs. 1.82 ng/mL; $p = 0.04$; Cohen's $d = 0.67$). No other differences were observed between the three sleep apnea severity groups in terms of plasma IL-6, TNFR1, leptin, or adiponectin in males or females.

Finally, we assessed the association of sleep apnea with a comorbid cardiovascular outcome (i.e. hypertension) by examining differences in inflammatory markers between three groups (non-sleep apneic/non-hypertensive controls, sleep apneics without hypertension, and sleep apneics with hypertension). Importantly, AHI did not differ between sleep apneics with or without

Table 1
Demographic and sleep characteristics of study sample, stratified by gender.

	Male			Female		
	Control (AHI < 5) ($n = 15$)	Sleep Apnea (AHI ≥ 5) ($n = 47$)	p	Control (AHI < 5) ($n = 23$)	Sleep Apnea (AHI ≥ 5) ($n = 35$)	p
Age, years	52.70 (1.52)	53.46 (0.86)	0.67	55.12 (1.19)	56.86 (0.97)	0.26
BMI, kg/m ²	26.50 (0.63)	27.79 (0.35)	0.08	28.37 (0.94)	31.09 (0.76)	0.03*
Waist circumference (cm)	96.03 (1.78)	100.54 (1.02)	0.03*	92.33 (2.47)	99.10 (2.00)	0.04*
Hip circumference (cm)	101.67 (1.66)	105.04 (0.95)	0.08	108.26 (2.33)	112.50 (1.90)	0.17
Neck circumference (cm)	38.32 (0.65)	39.87 (0.37)	0.04*	34.45 (1.12)	35.21 (0.91)	0.60
AHI	2.26 (4.37)	22.94 (2.47)	<0.001***	1.76 (2.88)	22.25 (2.33)	<0.001***
Systolic blood pressure (mmHg)	127.53 (4.15)	132.33 (2.37)	0.32	120.24 (3.42)	134.52 (2.73)	0.002**
Diastolic blood pressure (mmHg)	77.27 (2.23)	79.11 (1.27)	0.48	73.52 (1.97)	77.24 (1.57)	0.15
Total sleep time, min	368.77 (13.19)	351.54 (7.11)	0.80	368.24 (12.22)	363.87 (8.87)	0.68
Sleep efficiency, %	76.65 (2.79)	73.05 (1.48)	0.77	76.57 (2.52)	75.51 (1.82)	0.61
Sleep onset latency, min	21.23 (3.75)	20.00 (2.17)	0.64	25.47 (4.58)	30.90 (3.77)	0.93
Wake after sleep onset, min	95.90 (12.20)	114.14 (7.40)	0.83	90.43 (9.48)	92.00 (7.35)	0.60
Total wake time, min	112.50 (13.52)	129.73 (7.16)	0.77	112.43 (12.17)	117.97 (8.78)	0.62
Stage 1, %	27.02 (2.90)	30.75 (1.91)	0.51	22.43 (1.87)	24.97 (1.95)	0.80
Stage 2, %	58.26 (2.63)	52.61 (1.78)	0.47	53.18 (1.75)	50.78 (1.54)	0.39
Slow-wave sleep, %	4.06 (1.46)	4.81 (0.83)	0.65	12.75 (1.86)	12.88 (1.27)	0.44
Rapid eye movement sleep, %	10.67 (1.83)	11.83 (0.92)	0.73	11.63 (1.45)	11.37 (1.10)	0.83

Data are means (standard error of the mean). AHI = apnea hypopnea index. BMI = body mass index.

* $p < 0.05$

** $p < 0.01$

*** $p < 0.001$

Table 2
Inflammatory and metabolic markers in participants with and without sleep apnea, stratified by gender.

	Male		Female	
	Control (AHI < 5) ($n = 15$)	Sleep apnea (AHI ≥ 5) ($n = 47$)	Control (AHI < 5) ($n = 23$)	Sleep apnea (AHI ≥ 5) ($n = 35$)
IL-6 (pg/mL)	0.82 (0.16)	1.14 (0.09)	1.57 (0.28)	1.38 (0.22)
TNFR1 (ng/mL)	0.97 (0.06)	1.13 (0.04) [#]	1.23 (0.06)*	1.26 (0.05)
CRP (ng/mL)	0.90 (0.30)	1.56 (0.16)	1.83 (0.36)**	2.81 (0.28)** [#]
Leptin (ng/mL)	6.83 (0.91)	5.54 (0.50)	28.52 (3.31)***	32.96 (2.65)***
Adiponectin (ng/mL)	5.70 (0.76)	5.13 (0.42)	13.38 (1.49)***	11.53 (1.19)***

Data are means (standard error of the mean). Inflammatory and metabolic variables are adjusted for age and BMI.

* $p < 0.05$ for males vs. females.

** $p < 0.01$ for males vs. females.

*** $p < 0.001$ for males vs. females.

[#] $p < 0.05$ for controls vs. sleep apnea.

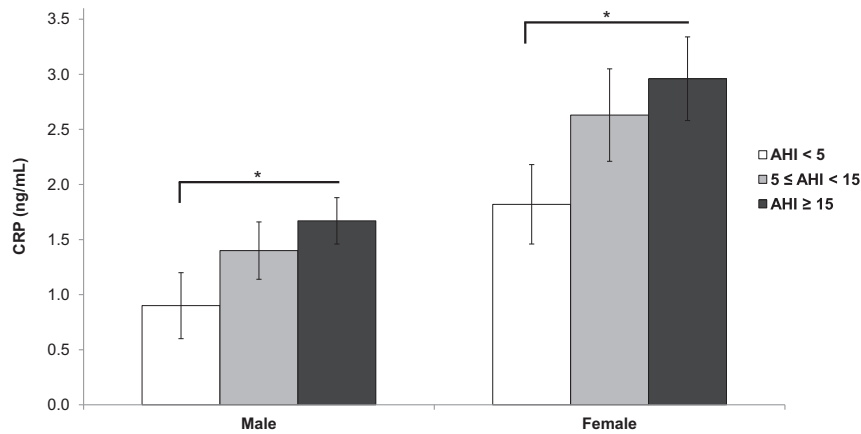


Fig. 1. C-reactive protein levels are elevated with increasing sleep apnea severity. Mean C-reactive protein concentrations in males and females, stratified by sleep apnea severity. Data adjusted for age and BMI. Error bars represent standard error of the mean. AHI = apnea/hypopnea index. CRP = C-reactive protein. * $p < 0.05$.

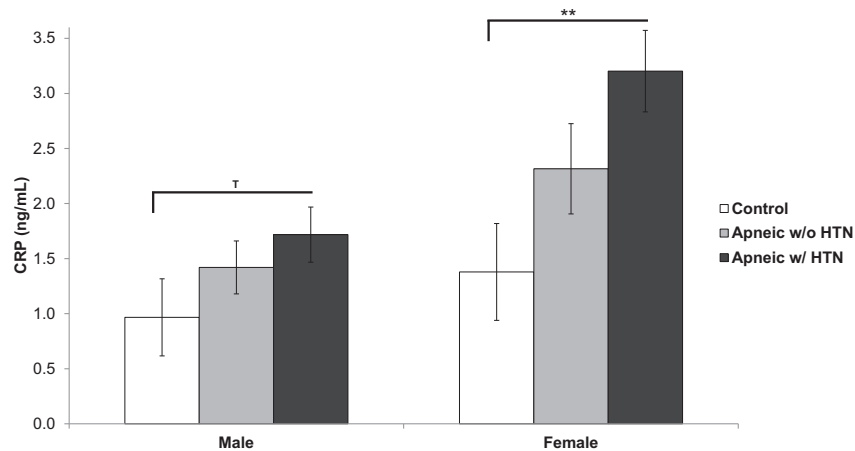


Fig. 2. Association of C-reactive protein with sleep apnea and comorbid hypertension in males and females. Mean C-reactive protein concentrations of controls, sleep apneics without hypertension, and sleep apneics with hypertension. Data adjusted for age and BMI. Error bars represent standard error of the mean. HTN = hypertension. ** $p < 0.01$, * $p < 0.1$.

hypertension in both men (AHI = 20.87 vs. 24.92 respectively; $p = 0.43$) and women (AHI = 20.80 vs. 24.44; $p = 0.49$). A dose-response was observed with CRP in both genders, although this association was stronger in women (p -linear = 0.005) than in men ($p = 0.09$) (Fig. 2). Sleep apneic women with hypertension ($n = 20$) had the highest CRP levels (3.20 ng/mL vs. 1.38 ng/mL in controls; $p = 0.005$; Cohen's $d = 1.09$). In men, sleep apneics with hypertension ($n = 23$) also had the highest CRP levels (1.72 ng/mL vs. 0.97 ng/mL in controls; $p = 0.09$; Cohen's $d = 0.64$). No other associations were observed between hypertension and any of the inflammatory or metabolic variables in either men or women.

4. Discussion

This is the first large study to examine gender differences in the profiles of inflammatory and metabolic markers in middle-aged, predominantly non-obese sleep apneics and controls. Although healthy women tend to have significantly higher values of TNFR1, CRP, leptin, and adiponectin than men, men with apnea appear to have a more severe inflammatory response, independent of age and body mass index. CRP is consistently elevated in both genders, and appears to be a significant predictor of apnea severity as well as comorbid cardiovascular problems in both men and women.

Our findings suggest that the inflammatory and metabolic abnormalities in sleep apnea should be examined separately in men and women, and CRP may be a clinically useful and reliable marker of sleep apnea severity and comorbid cardiovascular problems in both genders.

Previous work in the cardiovascular field supports our finding that peripheral CRP (Cartier et al., 2009; Khera et al., 2005; Lakoski et al., 2006; McConnell et al., 2002; Wener et al., 2002), leptin (Couillard et al., 1997; Hellström et al., 2000; Hickey et al., 1996; Kennedy et al., 1997; Ostlund et al., 1996), and adiponectin levels (Böttner et al., 2004; Saltevo et al., 2009; Song et al., 2014) are naturally higher in women compared to men, independent of age, race, and body mass index. Our study expands these findings in demonstrating that other inflammatory markers, such as TNF, are also higher in women than in men. Although gender differences in inflammatory and metabolic markers among healthy individuals have mostly been observed within pre-menopausal women and age-matched males, our findings indicate that these differences persist at least several years beyond menopause. All women in our study were post-menopausal, and the mean age of our control females was five years older than the widely cited median age (50 years) of menopause onset in American women (Kato et al., 1998; National Institute on Aging, 2008). These higher levels of inflammatory and metabolic markers in women compared to

men are likely related to the fact that women, independent of BMI, tend to have more body fat than men (Ley et al., 1992). Furthermore, previous work has demonstrated that in males and females matched for body fat content, women tend to have higher plasma concentrations of CRP (Khera et al., 2005) and leptin (Couillard et al., 1997; Kennedy et al., 1997) per unit fat mass. Interestingly, a recent study by Doran et al. (2013) in a large, representative U.S. population sample demonstrated that men with serum CRP > 3.0 mg/L have significantly higher odds of both cardiovascular and all-cause mortality, whereas neither outcome was observed in women with the same CRP cut-off. For these reasons, inflammatory and metabolic markers should be examined separately in men and women, and different cut-off points should be applied for the two genders.

In the present study, we found that men with sleep apnea showed significantly elevated TNFR1 levels and a trend toward higher CRP and IL-6 levels as compared to control men. In females, however, only CRP was significantly elevated in sleep apneics ($p < 0.04$). Together, these data suggest that, in middle-aged individuals, the inflammatory and metabolic aberrations in males with mild-to-moderate sleep apnea appear to be more severe than that of females. This can be explained by previous work showing that even within a relatively non-obese sample of males, visceral fat is the strongest predictor of sleep apnea, whereas total fat in females is most strongly associated with sleep apnea (Vgontzas et al., 2000; Kritikou et al., 2013). Due to the nature of visceral fat and its propensity to accumulate more in males (Lemieux et al., 1993), this may explain why men are more susceptible to a poorer metabolic and pro-inflammatory profile. Compared to subcutaneous fat, visceral fat has reduced sensitivity to insulin (Saltiel and Kahn, 2001) and is more vascularized, innervated, and contains a larger number of inflammatory and immune cells, making it more vulnerable to metabolic risks (Ibrahim, 2010). Although we did not examine specific fat types in the present study, it is likely that the sleep apneic men exhibited a greater proportion of this more harmful fat type than their female counterparts, accounting for the different inflammatory profiles between men and women. Interestingly, we did not observe significantly elevated leptin or reduced adiponectin in sleep apneics compared to controls in either males or females. Previous work by our group has demonstrated that obese and non-obese male sleep apneics have significantly higher plasma leptin levels (Kritikou et al., 2013, 2014; Vgontzas et al., 2000). However, in the present study, we examined only mild-to-moderate sleep apneics in contrast to more severe apnea, and only a single morning sample was used compared to twice a day or 24-h blood sampling in prior studies.

When we examined the association between inflammatory and metabolic markers with sleep apnea severity, only CRP emerged with a significant dose–response trend in both men and women (Fig. 1). In order to further assess the relationship of inflammatory markers and sleep apnea with clinically-meaningful adverse outcomes, we examined the association of the various inflammatory and metabolic markers with hypertension, controlling for severity of apnea. Once again, a significant dose–response association was observed most strongly with CRP relative to other inflammatory and metabolic markers. In females, independent of age and BMI, sleep apneics with hypertension had significantly higher levels of plasma CRP compared to controls and sleep apneics without hypertension. The same linear trend was observed in men, but was not as strong, due most likely to the higher variability of the values in the male control group (Fig. 2). Finally, the stronger and consistent association of CRP with severity of sleep apnea and associated cardiovascular morbidity, compared to the other markers, may reflect that CRP levels do not exhibit a circadian influence (Meier-Ewert et al., 2001).

No studies to date have explored the synergistic effect of apnea and hypertension on inflammatory and metabolic outcomes in men and women. Three of the larger, population-based studies in the field, in fact, reported no effect of gender on sleep apnea-associated hypertension prevalence in middle-aged participants (Bixler et al., 2000; Nieto et al., 2000; Peppard et al., 2000). In contrast, a population-based study has shown that men in the highest AHI tertile have a 3.7-fold risk of hypertension, whereas women in the highest tertile only have a 1.6-fold increased risk (Hedner et al., 2006). Furthermore, men within the highest BMI quartile have been shown to have a twofold higher risk for hypertension than BMI-matched women, independent of sleep apnea (Mohsenin et al., 2009). Another large prospective study reported a significantly increased stroke risk in the highest AHI quartile in men, but not women (Redline et al., 2010). Interestingly, there is also evidence of a gender difference in hypertension risk in response to intermittent hypoxia in an animal model; Hinojosa-Laborde and Mifflin (2005) demonstrated that female rats subjected to intermittent hypoxia were at a lower risk for developing elevated blood pressure than males. Interestingly, a recent study reported an association between blunted nocturnal blood pressure decline (“non-dipping”) and sleep apnea with elevated serum CRP, but this association was not observed in sleep apneics with a normal dipping pattern; however, this study did not analyze the genders separately (Ishikawa et al., 2008). Our results, showing a more severe inflammatory profile in men than women, suggest that future work should examine carefully the differential association of sleep apnea with cardiovascular complications between the two genders.

There are several limitations to our study that may impact its generalizability. First, the study was conducted in middle aged, mild-to-moderate sleep apneics who were relatively non-obese. Additional studies should explore the profiles of inflammatory and metabolic markers in individuals comprising a larger age range, within a larger sample of obese individuals, and in those with more severe sleep apnea. Second, we only performed a blood draw in the morning after awakening (7:00). We have previously demonstrated that plasma IL-6 has a pulsatile 24-h secretion (Vgontzas et al., 2005b), and TNF α (Uthgenannt et al., 1995), leptin (Langendonk et al., 1998), and adiponectin (Gavrila et al., 2003) also display circadian rhythmicity. Because this phenomenon is not detected in CRP (Meier-Ewert et al., 2001), combined with our consistent observation of elevated CRP with increasing apnea severity and cardiovascular problems, this suggests that CRP may be the best preclinical marker to assess the degree of inflammation in sleep apnea when only a single blood draw is possible. Finally, within some of our groups (e.g. control males), we have a small sample size, resulting in larger variability. While, overall, we examined a large sample of 120 participants, breaking them into discrete categories limited our statistical power. Despite this, Cohen's d calculations suggest that the effect size of both apnea severity (Fig. 1) and hypertension (Fig. 2) on plasma CRP levels are moderate to large in both genders (Cohen's d range = 0.64–1.09). Future studies should aim to recruit equal numbers of sleep apneics and controls.

In summary, our findings suggest that the inflammatory and metabolic abnormalities in sleep apnea should be analyzed and interpreted separately in men and women. Not only are there contrasting profiles between genders in controls vs. sleep apneics, but the impact of inflammation on cardiovascular risk also differs between men and women. Although healthy women have higher levels of plasma inflammatory and metabolic markers than their male counterparts, the inflammatory response to obstructive sleep apnea does not appear to be as severe in females as it is in males. While the women of our sample are post-menopausal, it is likely that the protective effects of female hormones may still be in effect

within these early stages. This is supported by work in a large population cohort suggesting that apnea in women peaks at age 65, roughly 15 years after the onset of menopause (Bixler et al., 2001). Finally, CRP, compared to other inflammatory markers, may be a clinically useful and reliable marker of apnea severity and comorbid cardiovascular problems in both men and women because its secretion is not influenced by circadian factors.

Conflict of interest

All authors report no biomedical financial interests or potential conflicts of interest.

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